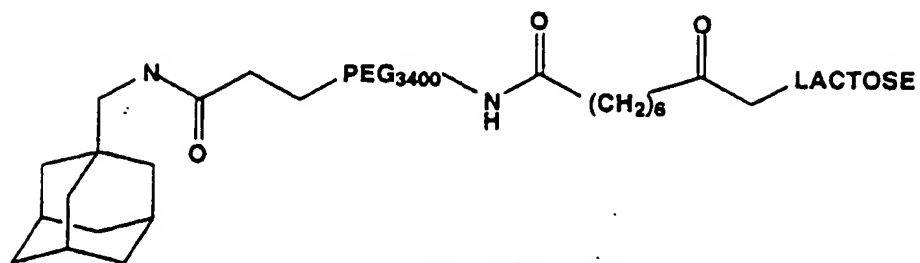
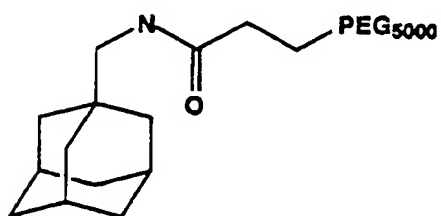


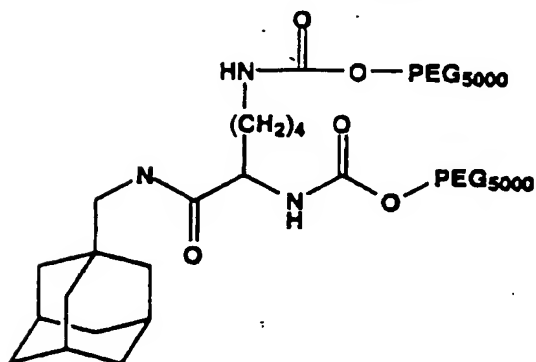
Ad-PEG<sub>3400</sub>



Ad-PEG<sub>3400</sub>-LAC

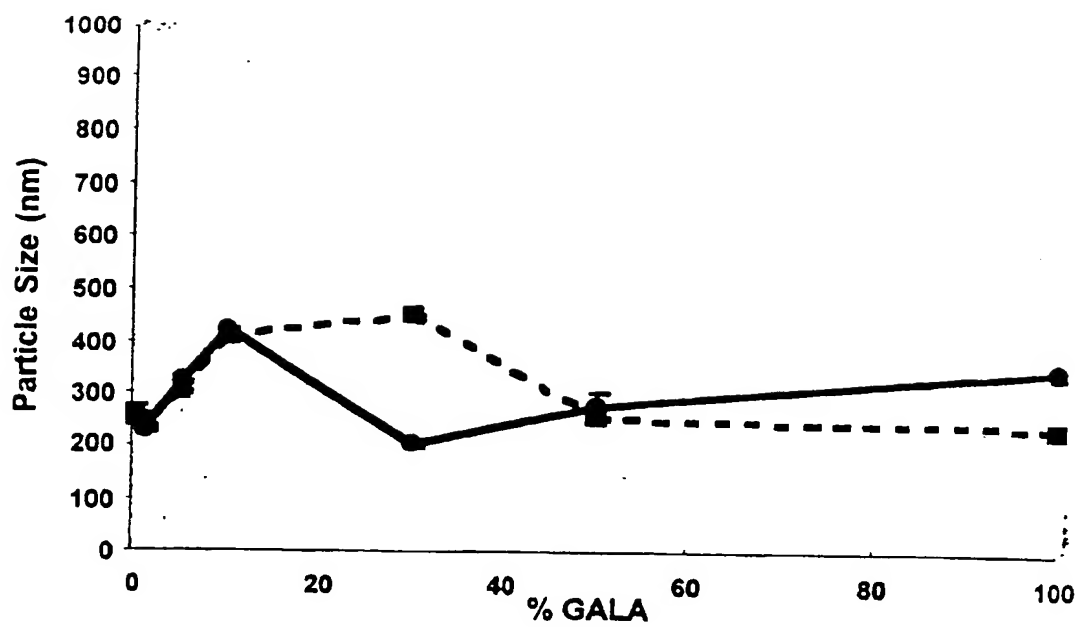


Ad-PEG<sub>5000</sub>

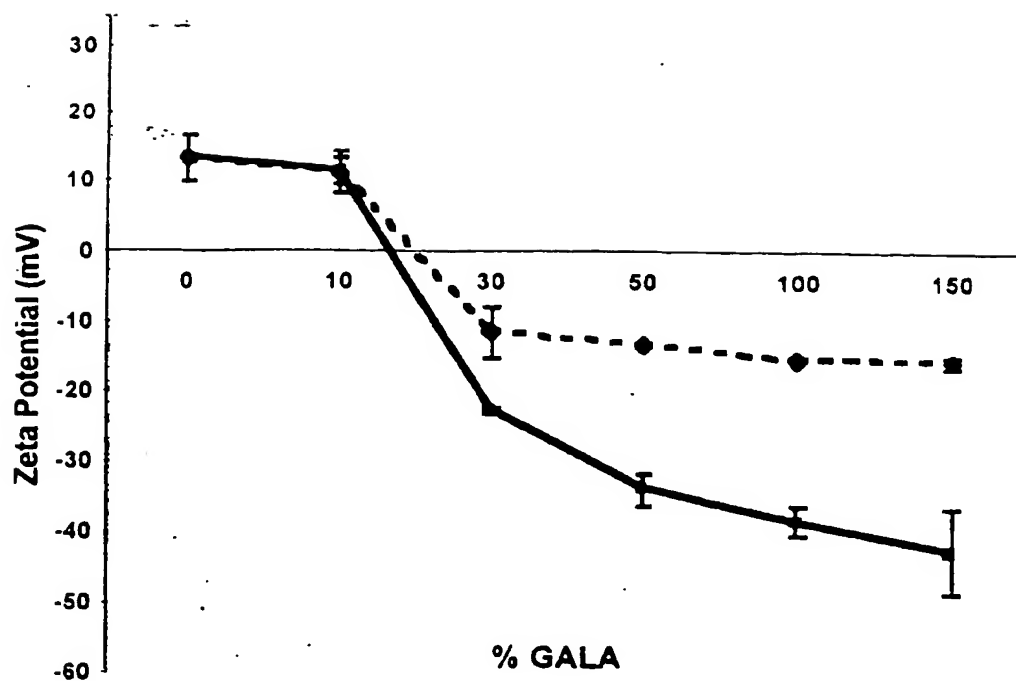


Ad-(PEG<sub>5000</sub>)<sub>2</sub>

Figure | Structures of Various Adamantane-PEG molecules.



**Figure 2.** Hydrodynamic diameter of GALA (dashed line) and GALA-Ad (solid line)-modified polyplexes.



**Figure 3.** Zeta potential of GALA (dashed line) and GALA-Ad (solid line)-modified polyplexes.

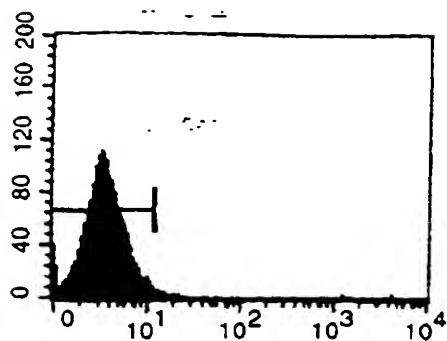


Fig 4a. Untransfected BHK-21 cells

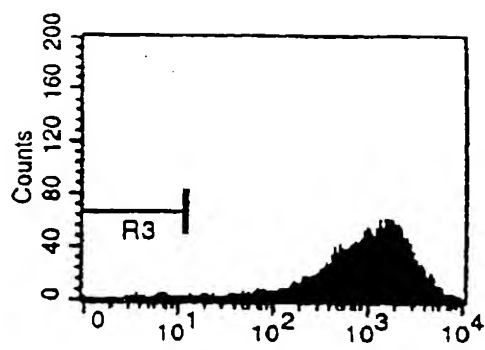


Fig 4b. BHK-21 cells transfected with  $\beta$ CDP6/FITC-Oligo at 5+/-

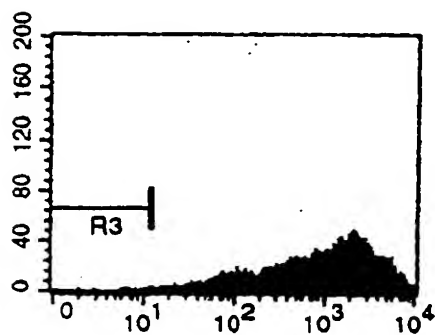


Fig 4c. BHK-21 cells transfected with  $\beta$ CDP6/FITC-Oligo/50% GALA

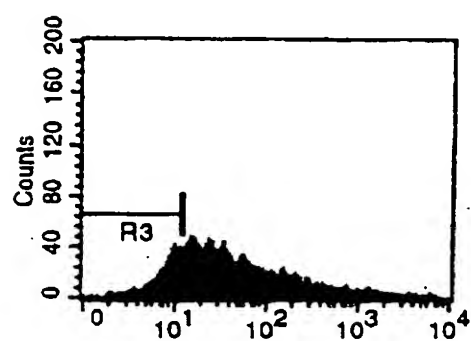


Fig 4d. BHK-21 cells transfected with  $\beta$ CDP6/FITC-Oligo/50% GALA-Ad

Fig 4. Uptake of GALA-Ad and GALA modified polyplexes by BHK-21 cells.

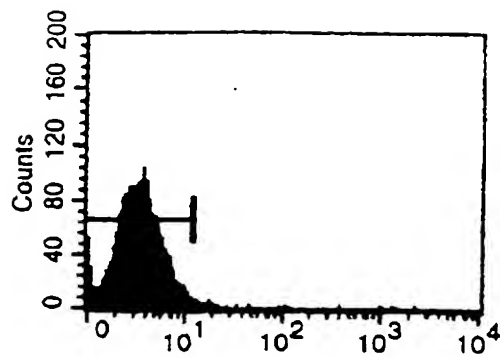


Figure 5a. Untransfected HUH-7 cells

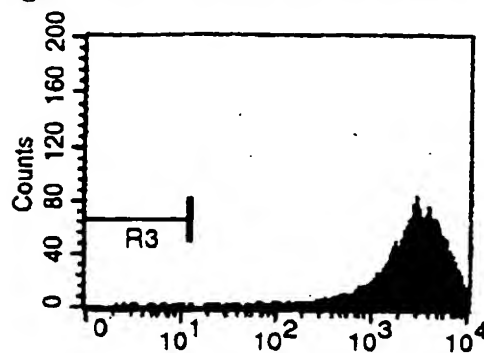


Figure 5b. HUH-7 transfected with  $\beta$ CDP6/FITC-Oligo at 5+/-

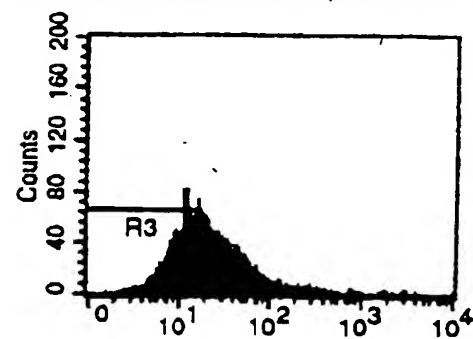
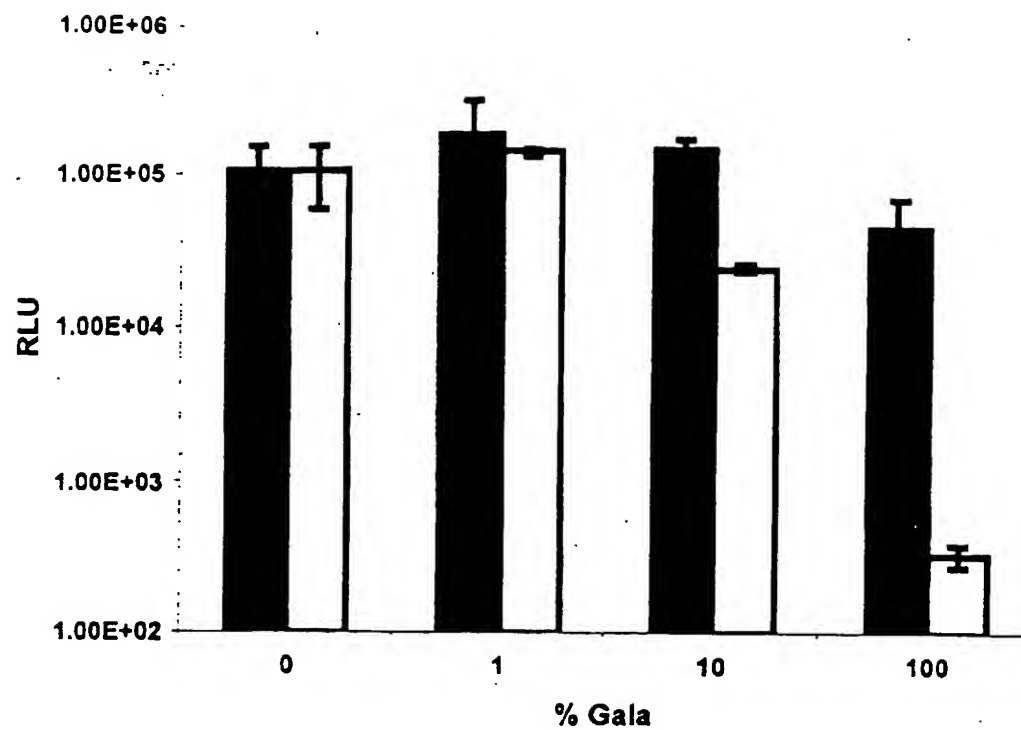
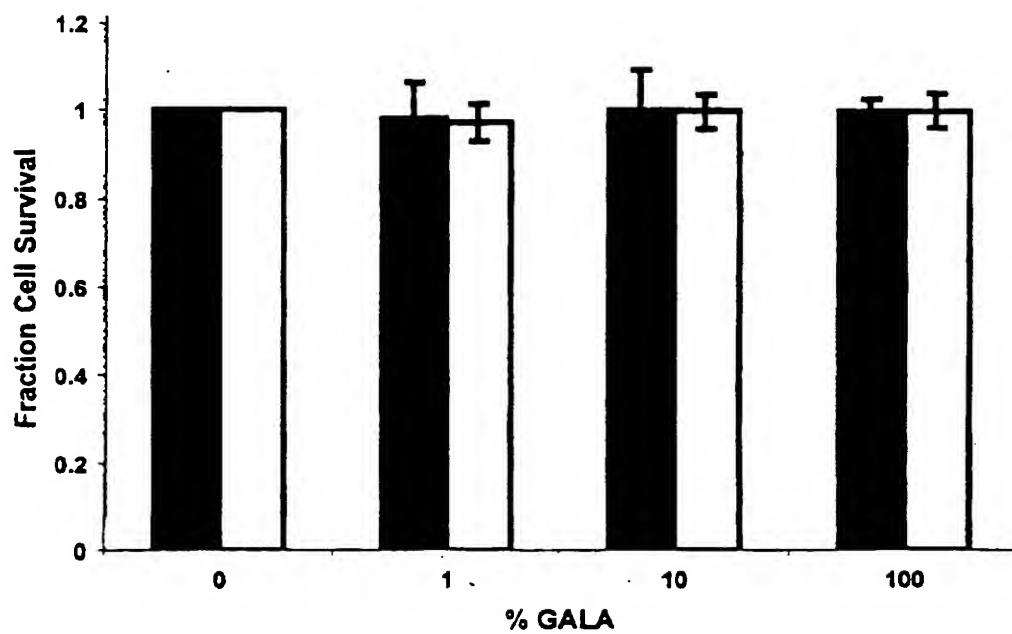


Fig 5c. HUH-7 transfected with  $\beta$ CDP6/FITC-Oligo/ 50% GALA-Ad

Fig 5. Uptake of GALA-Ad and GALA modified polyplexes by HUH-7 cells



**Fig 6.** Luciferase transfection of BHK-21 cells with  $\beta$ CDP-based polyplexes modified with GALA (shaded bars) and GALA-Ad (white bars).



**Fig 7. Toxicity of GALA and GALA-Ad modified polyplexes to BHK-21 cells.**

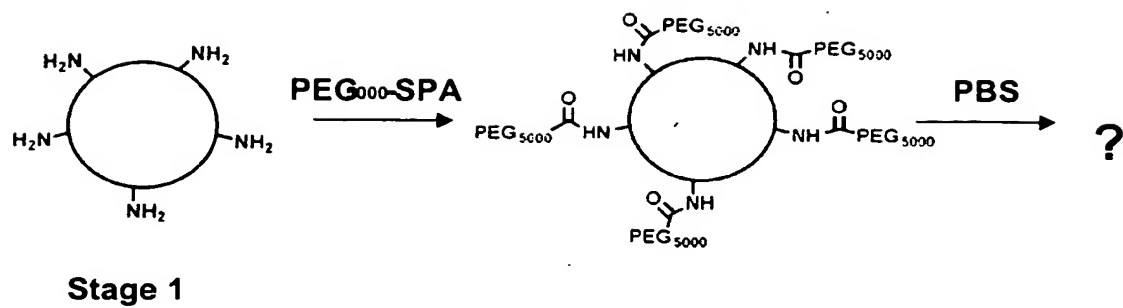


Figure 8: Scheme for post-DNA-complexation pegylation by grafting.



Polyplex	PEG	Stage 1 (nm)	Stage 2 (nm)	Stage 3 (nm)
PEI 3+/-	10:1	58	65	115
PEI 6+/-	10:1	55	60	78
$\beta$ CDP6 5+/-	100%	70	67.4	303
$\beta$ CDP6 5+/-	150%	70	X*	N/A
$\beta$ CDP6 5+/-	200%	70	X*	N/A
$\beta$ CDP6 5+/-	100% PEG**	67	81	700

\*Poor correlation function; no size measurements possible.

\*\*PEG<sub>5000</sub> added instead of PEG<sub>5000</sub>-SPA

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**Figure 9: Particle sizes of PEI and 12 ( $\beta$ CDP6) polyplexes during post-DNA-complexation pegylation of grafting.**

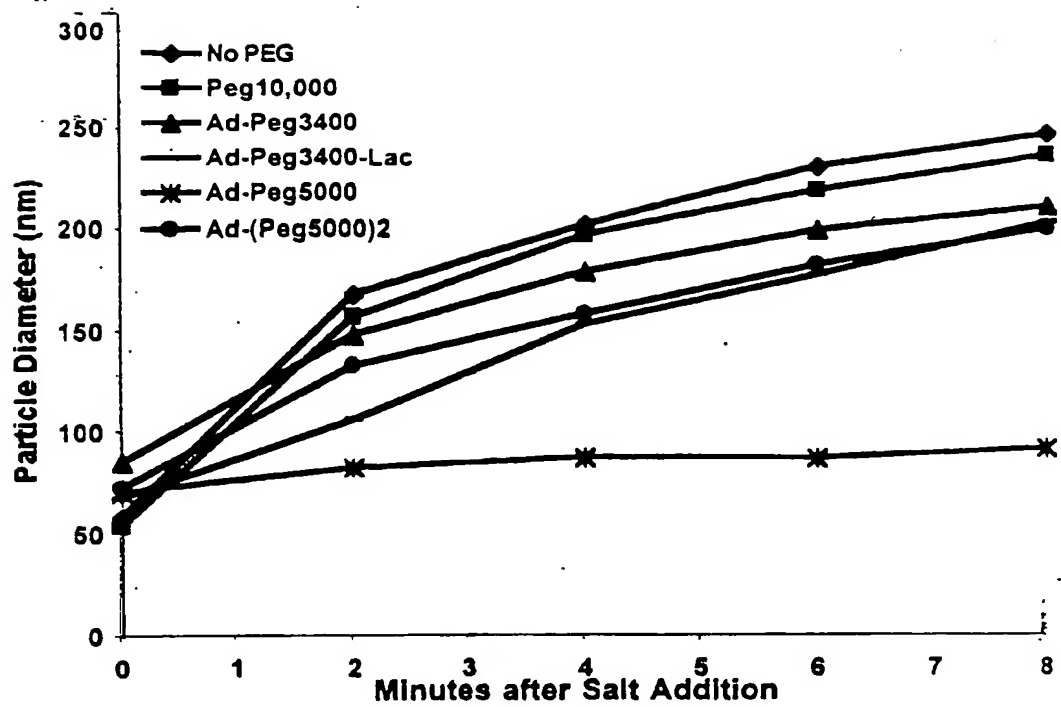


Fig 1 O Salt stabilization of polyplexes by pegylation.

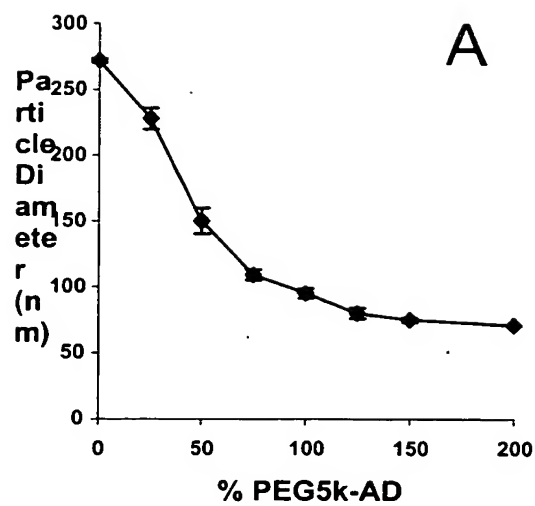


Figure 10A: Stabilization of polyplexes by pegylation.

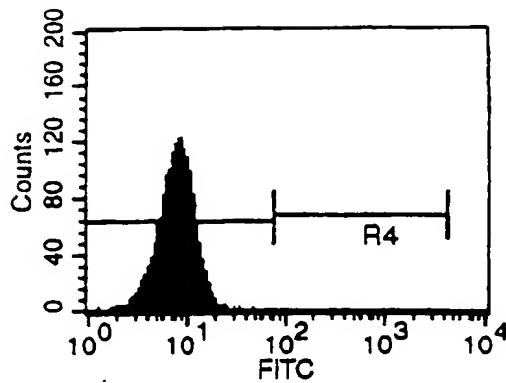


Fig 11a Untransfected HUH-7

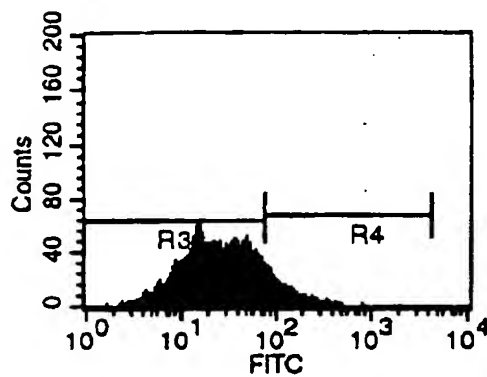


Fig 11b HUH-7 transfected with  $\beta$ CDP6/Oligo + free PEG<sub>3400</sub>-FITC

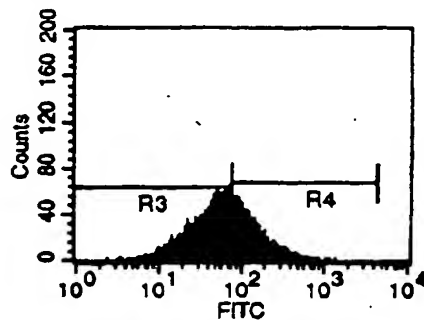
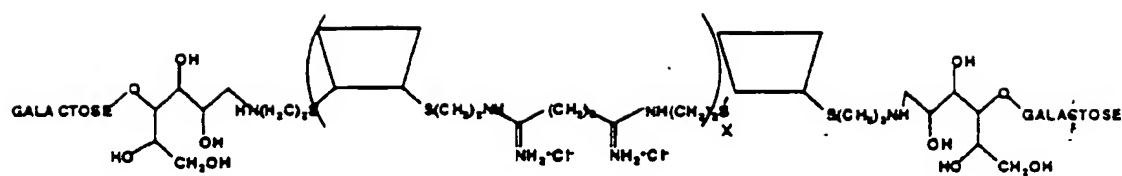
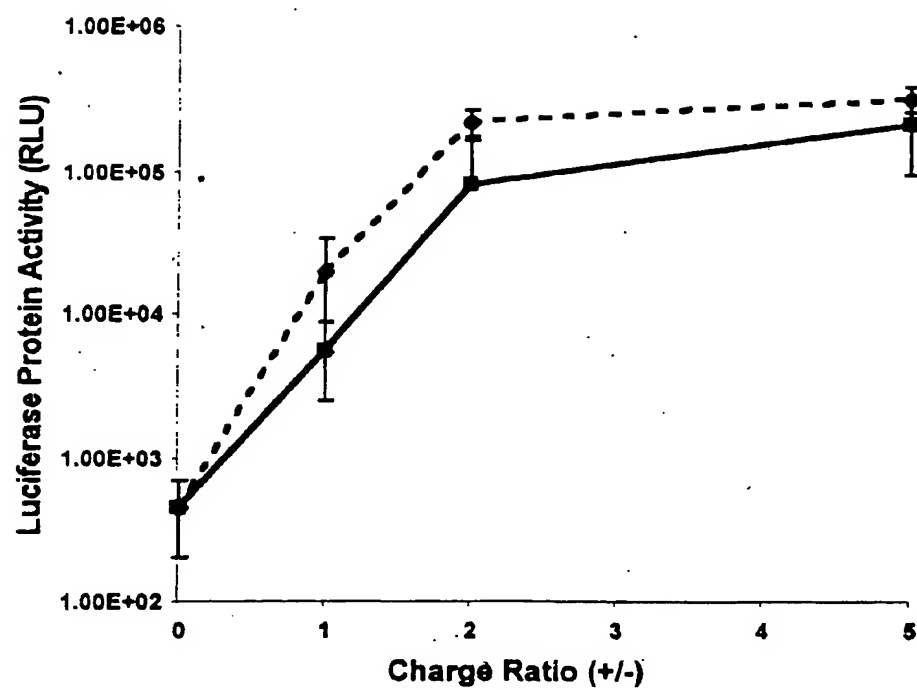


Fig 11c HUH-7 transfected with  $\beta$ CDP6/Oligo/Ad-PEG<sub>3400</sub>-FITC

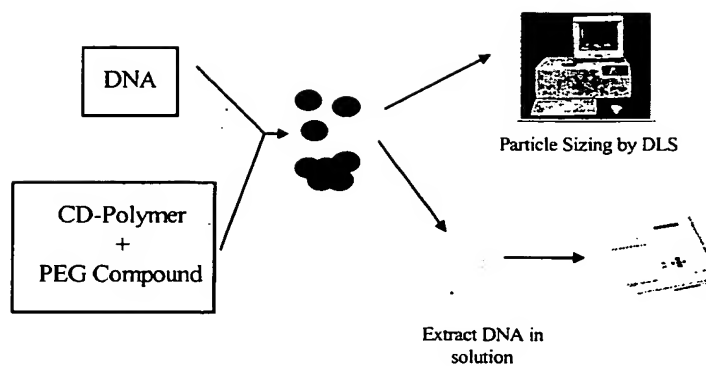
Fig 11 Co-delivery of  $\beta$ CDP6 polyplexes with PEG<sub>3400</sub>-FITC.



**Fig 12 Structure of Lactose-CDP6.**



**Fig 13** Transfection of  $\beta$ CDP (dashed line) and Lac-CDP6 (solid line) polyplexes to HUH-7 cells.



10 Figure 14. Schematic of Experimental Protocol, Example 47

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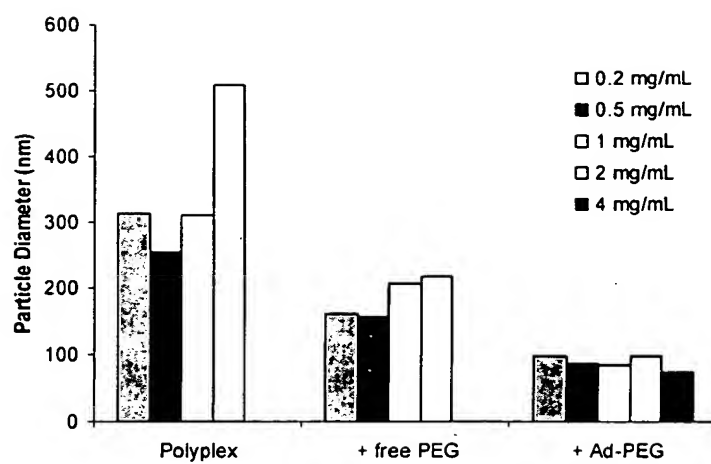


Figure 15. Particle Diameter



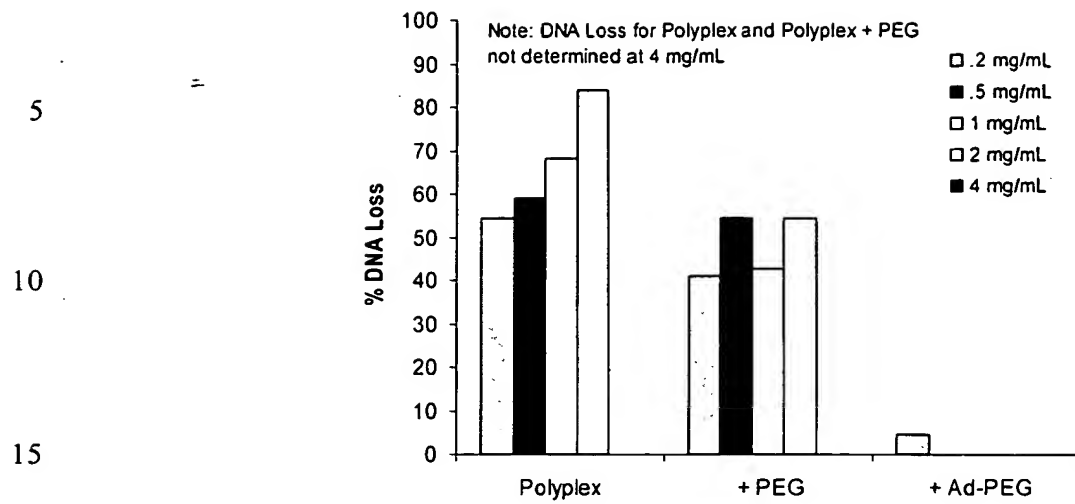
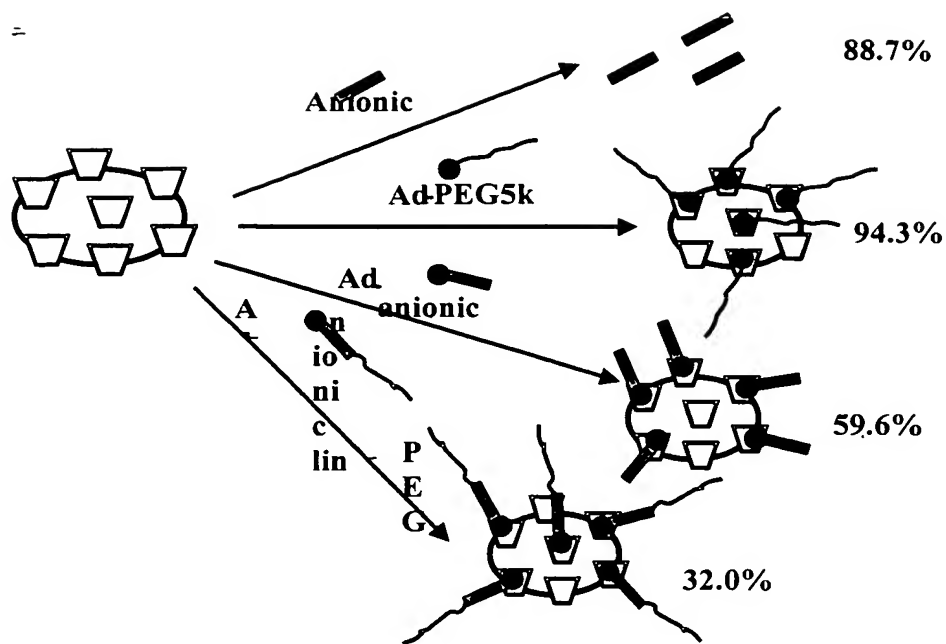
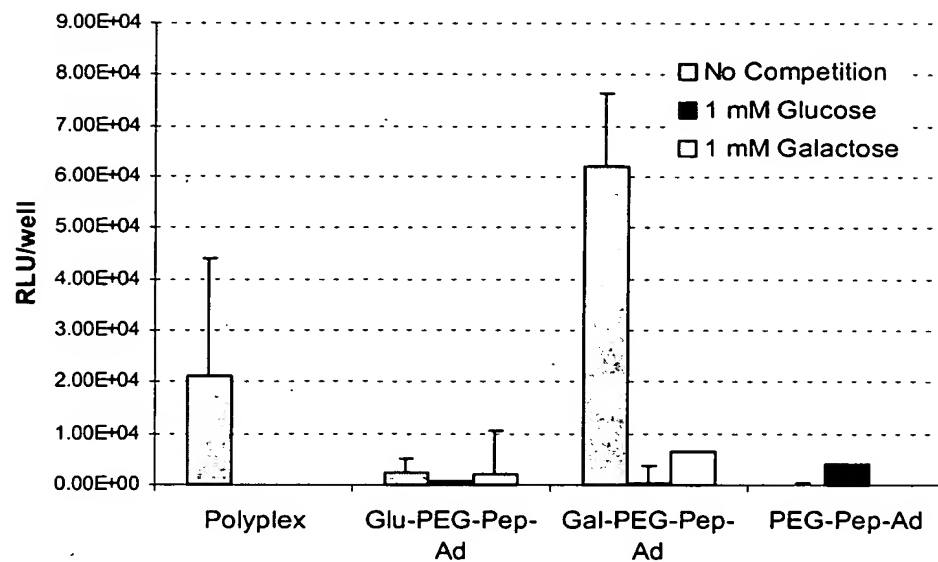


Figure 16. DNA Loss Due to Complex Precipitation



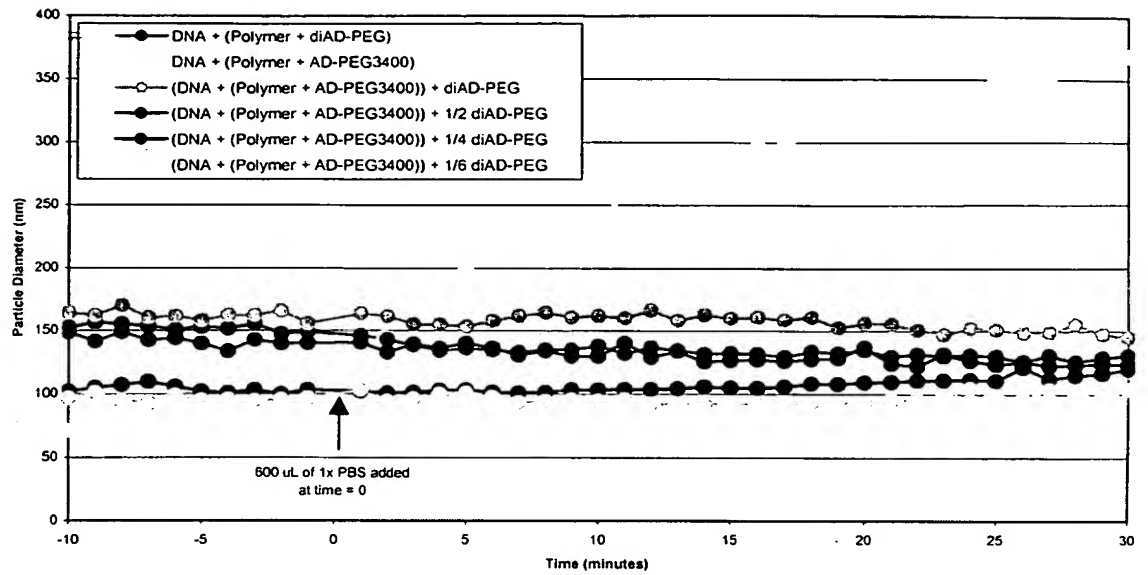
5 Figure 17 Inclusion Complexes to Modify 12/DNA Composite

# Transfection of Modified Polyplexes to HepG2 cells (50% PEG)



5

Figure 18



5 Figure 19 Competitive Displacement Experiments

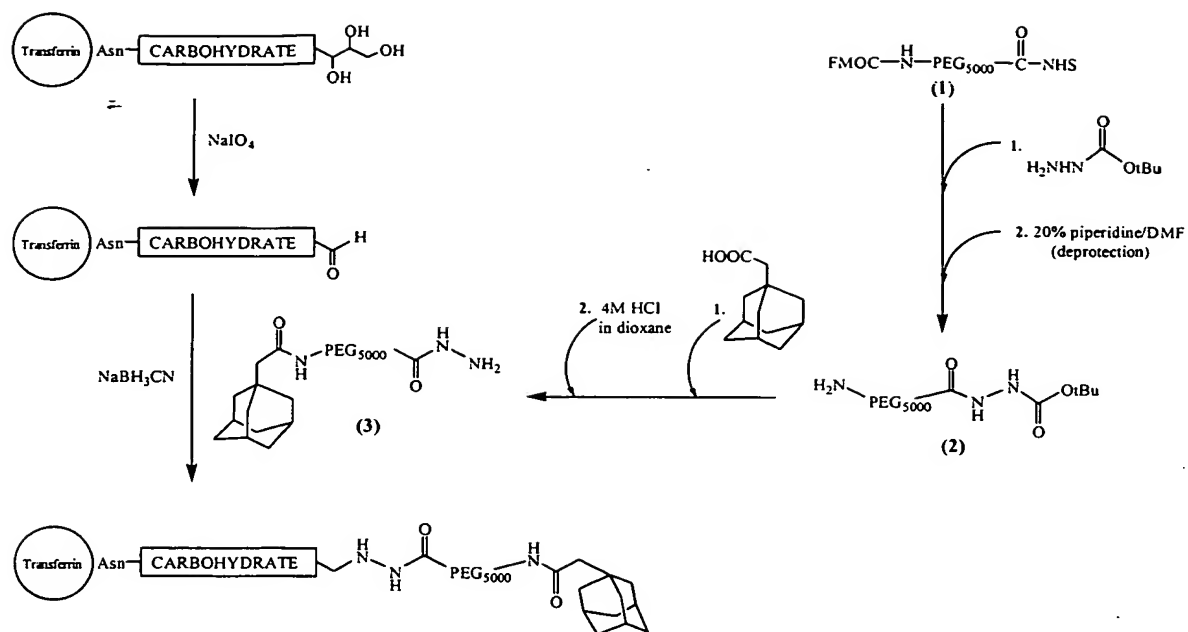


Figure 20 Synthesis of Adamantane-PEG-Transferrin (Ad-PEG-Tf)

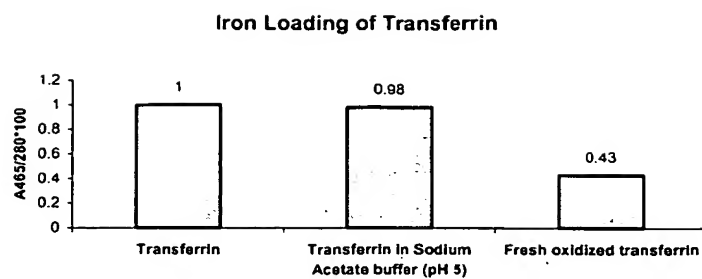


Figure 21

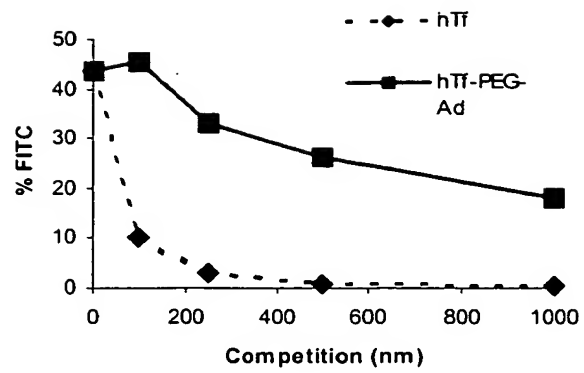
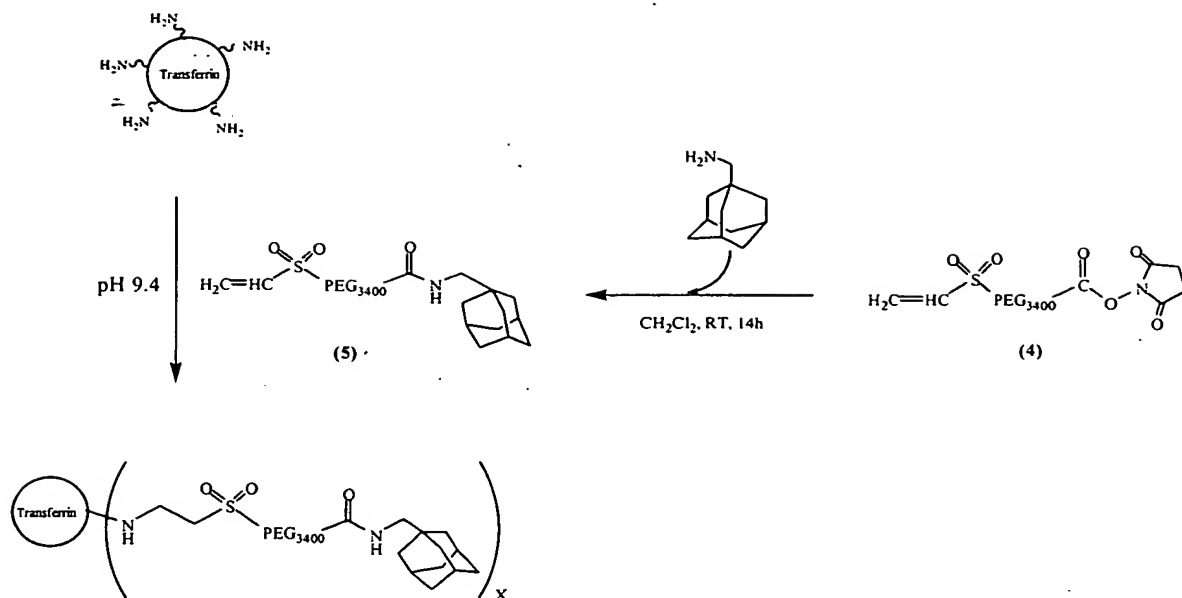


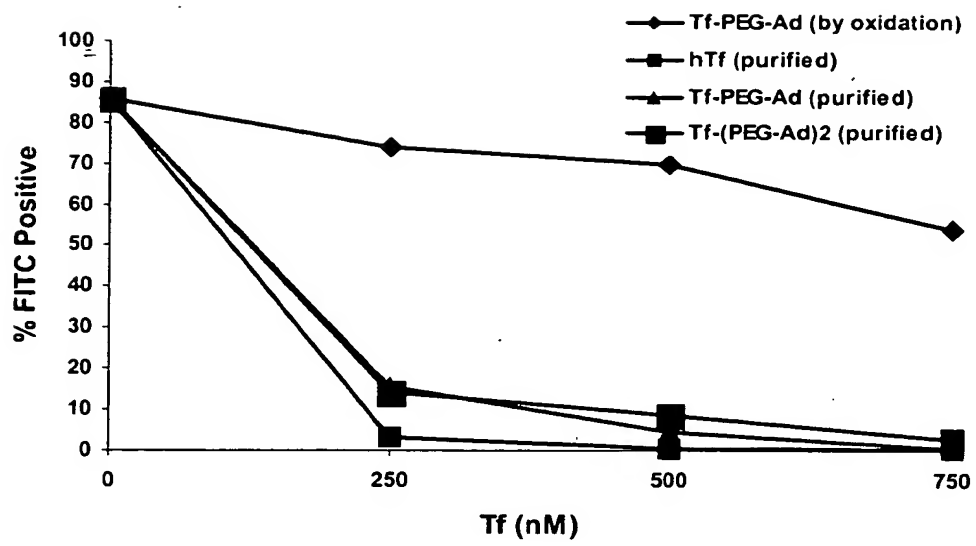
Figure 22 Binding Affinity Transferrin-PEG-Ad

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5 Figure 23 Transferrin coupling via Lysine groups





5 Figure 24 Binding affinity of Transferrin-PEG-AD to transferrin receptors on PC3 cells

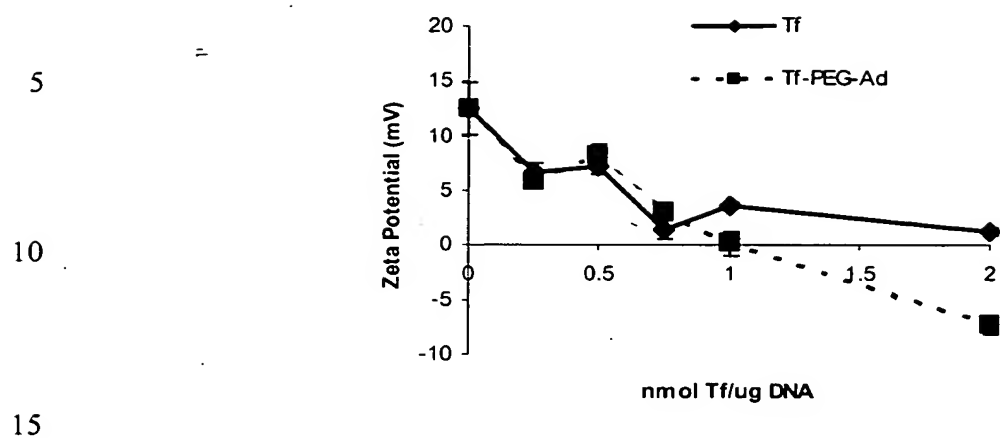


Figure 25 Zeta potential variation and particle size as a function of particle modification in transferrin and PEG-modified polyplexes

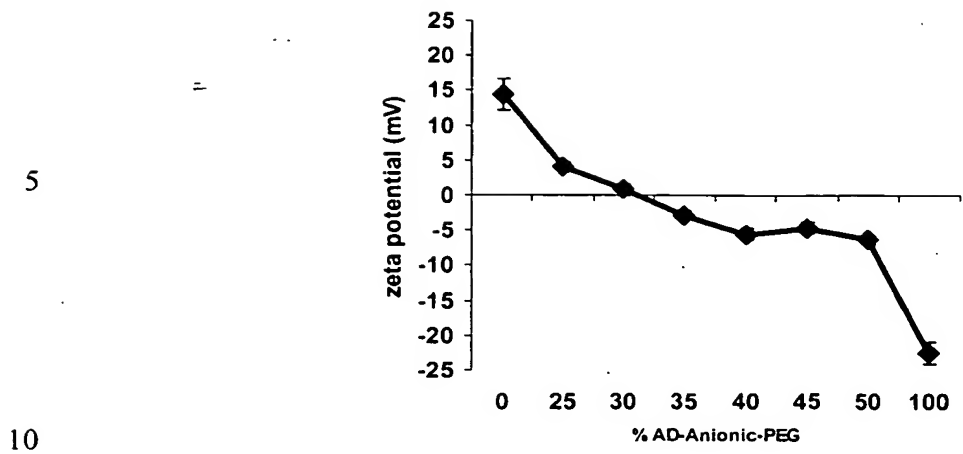


Figure 26 Zeta potential measurements, Ad-anionic-PEG

Stability in 150mM PBS using Ad-PEG  
1mg/ml DNA, 3+/- CDP

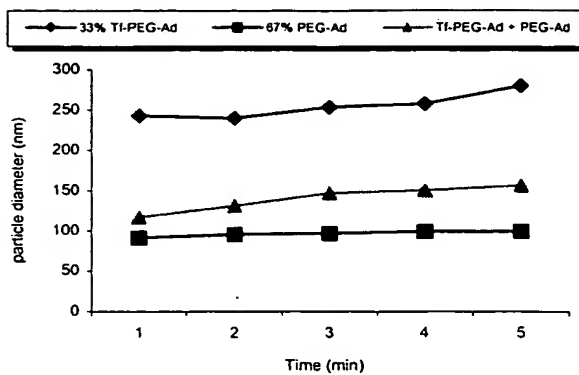


Figure 27

**Addition of increasing Tf-Modifier (balance is AD-PEG)  
1 mg/mL DNA, 3+/- CDP**

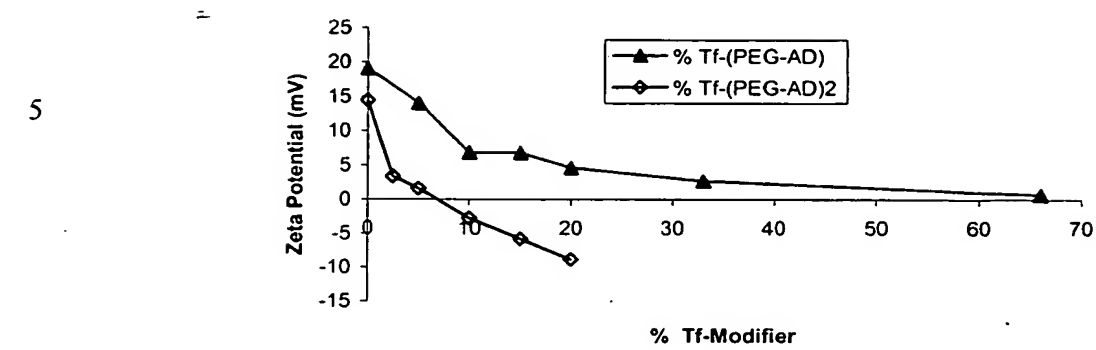
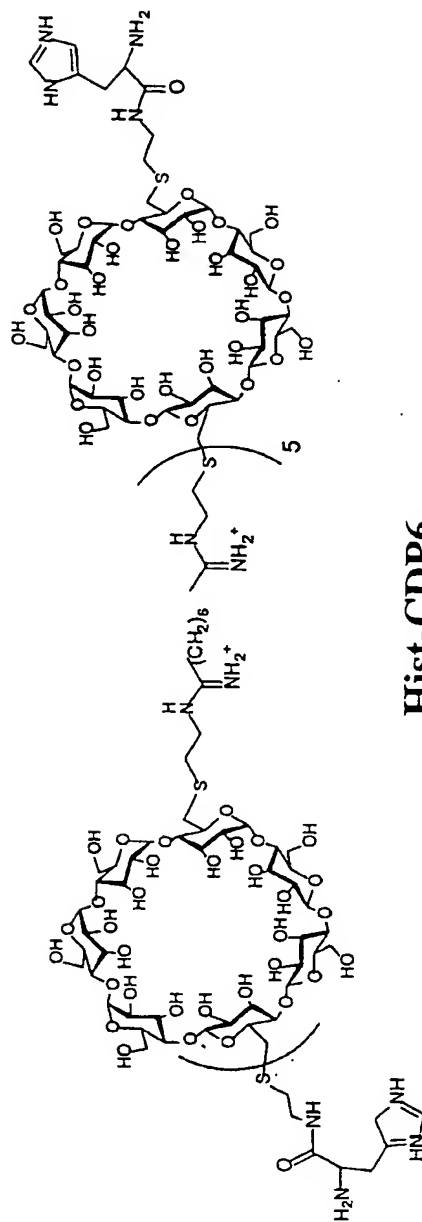
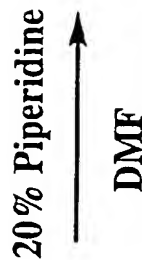


Figure 28

# Synthesis of Histidylated $\beta$ CDP6



**Hist-CDP6**

# pH-sensitive Polymers for Endosomal Escape

## Synthesis of secondary amine containing polymers

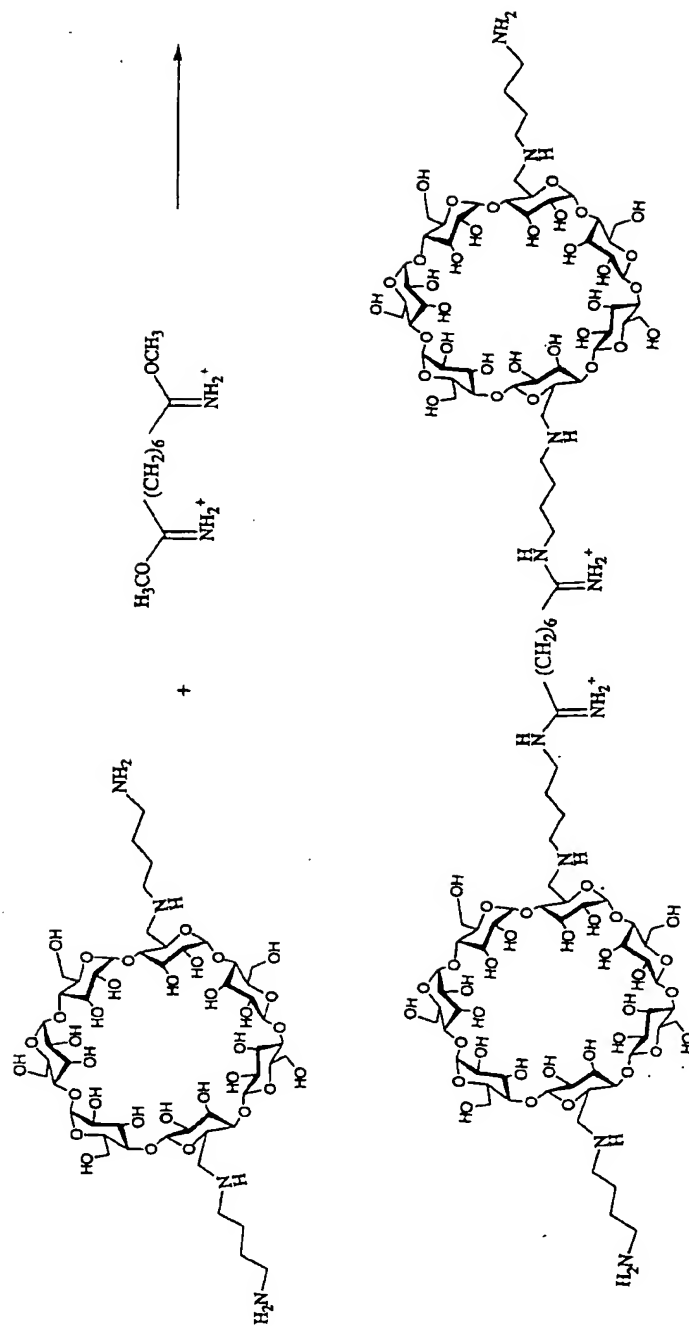


Figure 30